5-2015

Measuring Cannibalism Rates in the Zooplankton Copepod Species, Acartia tonsa

Katherine Shipman
College of William and Mary

Measuring Cannibalism Rates in the Zooplankton Copepod Species, *Acartia tonsa*

A thesis submitted in partial fulfillment of the requirement
for the degree of Bachelor of Science in Biology from
The College of William and Mary

by

Katherine Shipman

Accepted for Honors

Drew LaMar, advisor

Jonathan Allen

Harmony Dalgleish

Sarah Day

Williamsburg, VA
May 4, 2015

Acknowledgements

I would like to thank my committee members, especially my advisor Drew LaMar and Jonathan Allen for providing lab space. I would also like to thank David Elliott at VCU for helping with experimental set-up and procedures. I am grateful to Catherine King, Ann Rogers, Randy Chambers, and Sivan Yair for helping with the experiments. Lastly, I would like to thank the Roy R. Charles Center for funding my research through the William and Mary Honors Fellowship.

Abstract

Understanding the population dynamics of zooplankton is important to understanding the dynamics of the Chesapeake Bay, as zooplankton are at the interface of primary producers and higher trophic levels. Cannibalism is a potentially importance source of mortality for the copepod, *Acartia tonsa*, which is seasonally dominant in the Chesapeake Bay. In order to quantify cannibalistic behavior to improve terms and parameters in current zooplankton population models, I designed and conducted a two part experiment to measure cannibalism rates of *A. tonsa*. The experiments measured ingestion rates of phytoplankton, eggs and nauplii under varying starting concentrations of prey. Results were analyzed with 2-and 3-way ANOVAs and ingestion rates were fit with nonlinear Holling Type III models. This study adds to the current understanding of cannibalism in *A. tonsa* and highlights the need for further data collection.

Contents

1 Introduction **1**

2 Methods **3**
2.1 Zooplankton Collection 3
2.2 Phytoplankton Growth and Chlorophyll Estimation 4
2.3 Experiment 5
2.4 Estimation of Ingestion 7

3 Results **8**
3.1 ANOVA Analysis 8
3.1.1 2-Way ANOVA 8
3.1.2 3-Way ANOVA 13
3.2 Ingestion Fits 20
3.2.1 Single Cannibalism Prey 20
3.2.2 Two Cannibalism Prey 28

4 Discussion **30**

A Additional ANOVA Figures **34**
A.1 2-Way ANOVA 34
A.2 3-Way ANOVA 37

List of Figures

1.1 Life cycle diagram for *A. tonsa* 2

2.1 Pictures of egg traps. 4
2.2 Cultures of the phytoplankton *Rhodmonas lens.* 5
2.3 Plankton wheel set-up with sample bottles. 6

3.1 Egg Cannibalism ANOVA plots 10
3.2 Nauplii Cannibalism ANOVA plots 12
3.3 Phytoplankton ingestion versus phytoplankton concentration at various concentrations of egg and nauplii 15
3.4 Egg ingestion versus egg concentration at various concentrations of phytoplankton and nauplii 16
3.5 Nauplii ingestion versus phytoplankton concentration at various concentrations of eggs and nauplii 17
3.6 Nauplii ingestion versus egg concentration at various concentrations of phytoplankton and nauplii 18
3.7 Nauplii ingestion versus nauplii concentration at various concentrations of phytoplankton and eggs 19
3.8 Egg Cannibalism Individual Ingestion Fits 22
3.9 Egg Cannibalism: total ingestion 23
3.10 Nauplii Cannibalism Individual Ingestion Fits 26
3.11 Nauplii Cannibalism: total ingestion 27

A.1 Egg Cannibalism ANOVA plots 35
A.2 Nauplii Cannibalism ANOVA plots 36
A.3 Phytoplankton ingestion versus phytoplankton concentration at various concentrations of egg and nauplii 37
A.4 Egg ingestion versus egg concentration at various concentrations of phytoplankton and nauplii 38
A.5 Nauplii ingestion versus phytoplankton concentration at various concentrations of eggs and nauplii 39
A.6 Nauplii ingestion versus egg concentration at various concentrations of phytoplankton and nauplii 40

A.7 Nauplii ingestion versus nauplii concentration at various concentrations of phytoplankton and eggs 41

List of Tables

2.1 Values for biomass conversions for *A. tonsa* individuals based on stage class. 4
2.2 Concentrations used for each prey type in single prey cannibalism experiments. 6
2.3 Concentrations used for each prey type in two prey cannibalism experiments. 7

3.1 Egg Cannibalism ANOVA Results 9
3.2 Nauplii Cannibalism ANOVA Results 11
3.3 3-Way ANOVA Results 14
3.4 Egg Cannibalism Individual Ingestion Fits Parameter Values 21
3.5 Egg Cannibalism: total ingestion fit parameter values. 21
3.6 Nauplii Cannibalism Individual Ingestion Fits Parameter Values . . 24
3.7 Nauplii Cannibalism: total ingestion fit parameter values. 25
3.8 Two Cannibalism Prey Individual Ingestion Fits Parameter Values . 28
3.9 Two Cannibalism Prey Total Ingestion Fit Parameter Values 29

Chapter 1

Introduction

Zooplankton are near the base of the marine food web, directly above phytoplankton. They are the primary grazers of phytoplankton and are a food source for higher trophic levels, including fish and jellies. *Acartia tonsa* is a dominant species of zooplankton in the Chesapeake Bay, especially in the summer months. Therefore, studying its dynamics is important to understanding the dynamics of the Chesapeake Bay. In past research, the mortality parameter was determined to be the most sensitive parameter in linear *A. tonsa* population models [11], and it is usually given as a simple increasing linear function of temperature [5]. This indicates a need for more accurate and mechanistic representations of mortality within models. The research on different types of mortality is limited, but current literature suggests that cannibalism can be a major contributor.

A. tonsa is an omnivorous calanoid copepod that can survive in a wide range of salinities and temperature [14]. It has 13 stages of development, mostly determined by size, with their own development and mortality rates which are temperature dependent. There is an egg class, 11 juvenile classes, and an adult class. The juvenile classes are further divided into 6 nauplii classes and 5 copepodite classes. The adult class is the only class which can reproduce (see Figure 1.1), and it has been shown that adults will cannibalize eggs and juveniles [4, 14]. Adult females survive longer than males, generally have higher grazing rates [6, 8], and can be distinguished from males by difference in antennae shape. Eggs are released from the female post fertilization.

Adult *A. tonsa* have the ability to move and maintain their position within the water column. They have two feeding modes. One mode is filter feeding for ingesting phytoplankton and other small, non-motile prey. The other mode is "jumping" or ambush mode in which they attack motile prey from below in the water column [12]. Their feeding is size restricted, meaning they can only eat prey below a certain size threshold, which is common is marine food webs. This threshold increases

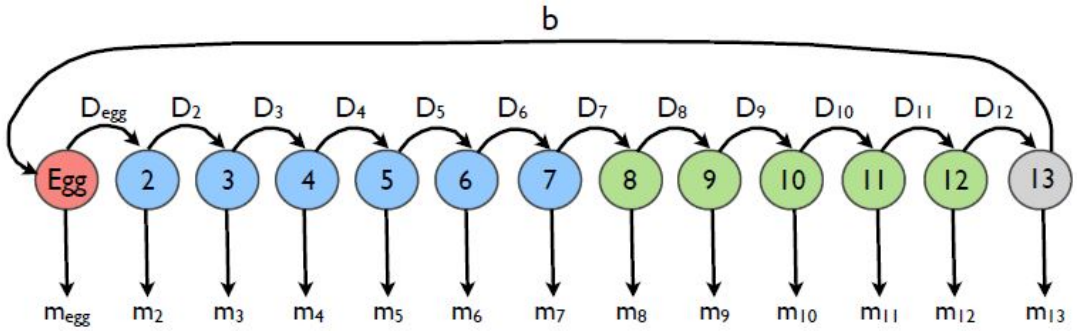


Figure 1.1: Life cycle diagram for *A. tonsa*. Blue circles represent nauplii. Green circles represent copepodites. The gray circle represents the adults.

through development and is based on body length [1].

A. tonsa suffer from three general types of mortality: non-predatory, which includes disease and starvation; higher trophic level predation; and intra-guild predation which includes cannibalism. From literature it was found that non-predatory mortality accounts for 30% of total mortality [5] and higher trophic level predation account for 10% [15]. This leaves up to 60% of the total mortality being due to intra-guild predation. While these proportions are unknown locally for the Chesapeake Bay, since *A.tonsa* is the by far the most dominant species in the summer, it is possible that cannibalism is the dominant form of intra-guild predation, and therefore an important source of mortality. Adults typically cannibalize eggs and the first two nauplii stages.

Of the previous studies on cannibalism of eggs and nauplii, one study showed that egg cannibalism was primarily affected by egg concentration followed by phytoplankton concentration [4]. Another study showed a significant positive relationship between cannibalism of nauplii and naupliar density and a significant negative relationship between cannibalism of nauplii and phytoplankton density [14]. Both studies indicate interaction effects between cannibalism prey density and phytoplankton density. However these studies do not include data on phytoplankton ingestion and do not include both nauplii and eggs, so it is hard to develop an accurate sense of prey choice. It is important to create accurate parameters for use in mechanistic nutrient-phytoplankton-zooplankton (NPZ) models.

The purpose of this study is to quantify cannibalistic behavior of adult *A. tonsa* individuals on eggs and nauplii. To generate an accurate model of cannibalism and prey choice, egg, nauplii and phytoplankton ingestion rates were measured over varying concentrations of all three in a three-way factorial design.

Chapter 2

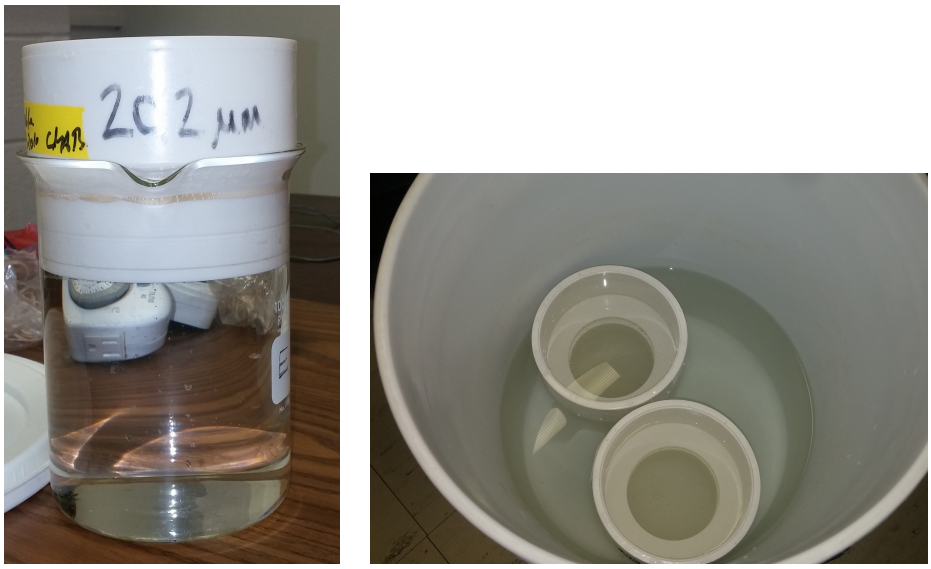
Methods

2.1 Zooplankton Collection

Adult *Acartia tonsa* were collected in July and August from the York River near Gloucester Point, VA which has a salinity near 22 ppt. The copepods were collected with a plankton net that was manually towed from a dock. Water was also collected from this location which was filtered for use throughout the experiments, so that the copepods could be maintained in similar conditions to those in their natural environment. Prior to the beginning of experimentation, the copepods were maintained at the laboratory under the same temperature and salinity conditions as used in the experiment and fed every other day from laboratory cultured *Rhodamonas lens*. The laboratory was maintained at a temperature around 21.0°C.

Eggs and nauplii were collected in the lab. Egg traps were constructed by keeping adult copepods separated from water below, thus allowing eggs to fall through $202\mu m$ mesh and sink to the bottom (see Figure 2.1). Adults were removed and eggs collected after 24 hours. For nauplii collection, the eggs were allowed to hatch and collected after an additional 12 to 24 hours after adult removal.

Throughout this paper all units will be in terms of biomass (μg C), so that data can easily be compared with the multiple prey types. The values used for *A. tonsa* biomass conversion are given in Table 2.1.



(a) Side view (b) Top view

Figure 2.1: Pictures of egg traps.

	Biomass (μg C/ind.)
Adult	4.92 [7]
Nauplius	.088 [3]
Egg	.0536 [10]

Table 2.1: Values for biomass conversions for *A. tonsa* individuals based on stage class.

2.2 Phytoplankton Growth and Chlorophyll Estimation

Rhodomonas lens was cultured in the laboratory in the presence of light 24 hours a day to speed growth using filtered water from the York River (see Figure 2.2). The algae was used to feed the zooplankton prior to experimentation and as an alternative food source during the experiments. The value used for *R. lens* biomass conversion is $5 \times 10^{-5}\mu$g C/cell [17].

Phytoplankton abundance was estimated based on chlorophyll measured with a spectrophotometer. Since the phytoplankton was incubated under the same conditions, we assume that the amount of chlorophyll per cell is the same. For calibration to determine the amount of chlorophyll per cell, cell counts were performed, followed by chlorophyll measurement of the algae culture.

For the cell counts, 1% Lugol's solution was added to a small volume of phy-

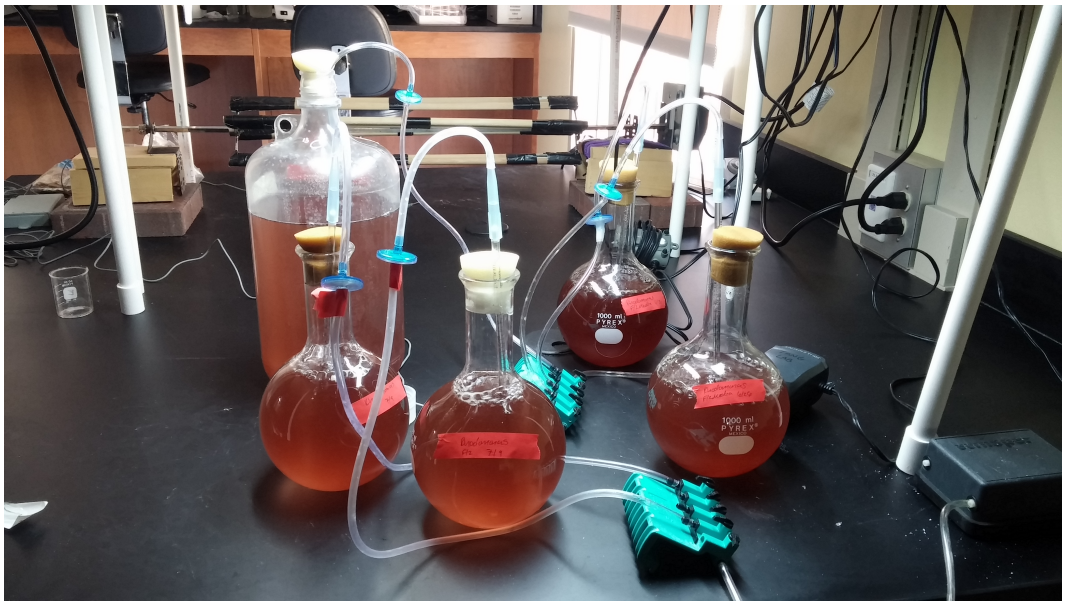


Figure 2.2: Cultures of the phytoplankton *Rhodmonas lens*.

toplankton to the fix the cells. The cells were counted using a counting chamber and a compound light microscope to determine the number of cells per milliliter.

To measure chlorophyll abundance, a small volume of phytoplankton was vacuum filtered onto GF/F filter paper. The filter paper was place into 10 mL of 90% acetone buffered with $MgCO_3$ in a dark freezer overnight to extract chlorophyll from cells. The absorbance of the chlorophyll sample was measured with a spectrophotometer at three wavelengths: 664 nm, 647nm, and 630 nm. A blank of 90% acetone was used to zero the machine. These values were used to calculate μg Chl_a/L with the following equation [9]:

$$Chl_a = 11.85(A_{664}) - 1.54(A_{647}) - 0.08(A_{630}). \tag{2.1}$$

With this information and the number of cells per milliliter, the amount of chlorophyll per cell was estimated.

2.3 Experiment

Each experimental unit for the feeding experiment consisted of a 70 mL tissue flask bottle with a vented cap filled with filtered York River water, two adult female *A.tonsa* individuals, and the appropriate amount of cannibalism prey and phytoplankton based on experimental concentrations described in Tables 2.2 and 2.3. Phytoplankton was added first followed by egg and/or nauplii, with adults

added last. Immediately after the adults were added, the sample bottle was placed on a plankton wheel, shown in Figure 2.3, for the experimental duration of 24 hours with a 12 hour light/dark cycle.

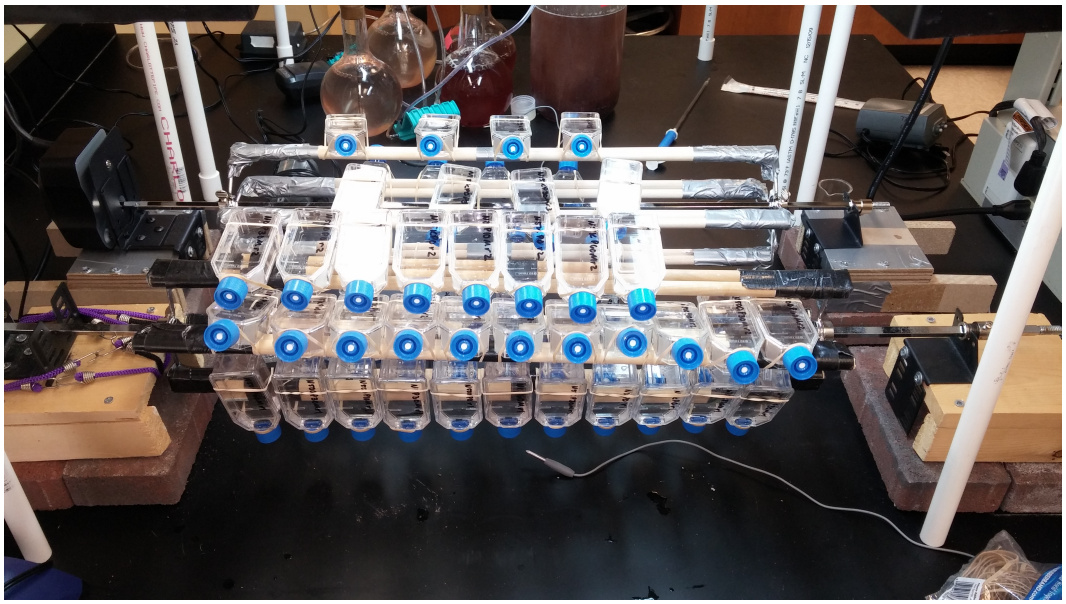


Figure 2.3: Plankton wheel set-up with sample bottles.

Cannibalism was first studied with only one cannibalism prey type: eggs or nauplii. In addition to the single cannibalism prey, the phytoplankton *R. lens* was used as an alternative food source. The concentrations of both were varied creating a factorial experiment. The concentrations used are listed in terms of biomass and in terms of individuals in Table 2.2. The concentrations were chosen in an attempt reflect the natural abundance of each prey type. However, they were limited by the number of eggs produced and nauplii that hatched. Each concentration combination had three replicates.

Prey	Biomass (μg C/L)	Individual (1/L)
Phytoplankton	0, 500, 1500, 3000	0, 1×10^7, 3×10^7, 6×10^7
Egg	0, 5.36, 93.8, 455.6	0, 100, 1750, 8500
Nauplii	0, 2.64, 36.96, 96.8	0, 30, 420, 1100

Table 2.2: Concentrations in terms of biomass as μg C per liter and in terms of individuals or cells (phytoplankton) per liter used for each prey type in single prey cannibalism experiments.

Following the study of cannibalism with one cannibalism prey, it was then studied with both eggs and nauplii together. In addition to the two cannibalism preys,

the phytoplankton *R. lens* was used as an alternative food source. The concentrations of all three were varied creating a factorial experiment. The concentrations used are listed in terms of biomass and in terms of individuals in Table 2.3. The concentrations of the two cannibalism prey experiment were initially meant to be similar to those of the single cannibalism prey experiments, but an increased number of samples due to the three-way factorial design and the limit on the amount of eggs produced by an adult resulted in lower final concentrations. Each concentration combination had two replicates instead of three due to a malfunction in one plankton wheel preventing proper mixing within the bottles.

Prey	Biomass (μg C/L)	Individual (1/L)
Phytoplankton	0, 250, 1000, 2500	0, 5×10^6, 2×10^7, 5×10^7
Egg	0, 2.68, 53.6, 187.6	0, 50, 1000, 3500
Nauplii	0, 1.32, 4.4, 28.16	0, 15, 50, 320

Table 2.3: Concentrations in terms of biomass as μg C per liter and in terms of individuals or cells (phytoplankton) per liter used for each prey type in two prey cannibalism experiments.

2.4 Estimation of Ingestion

Cannibalism and phytoplankton ingestion rates were measured indirectly by calculating the difference between initial abundances and remaining eggs, nauplii, and phytoplankton, with controls to correct for non-cannibalism mortality and development of eggs and nauplii and growth of phytoplankton. Ingestion results are reported per adult per day.

After each sample bottle was on the plankton wheel for 24 hours with 12-hour light/dark cycle, the bottles were removed. Each bottle was visibly checked for two living adults. The contents of the bottle were poured through a 64 μm mesh filter to collect the remaining eggs and nauplii. The filter was rinsed into a labeled petri dish with a few drops of Lugol's solution to preserve the sample until it could be counted. The eggs and nauplii were counted with a dissecting microscope and observations noted. The filtrate was collected and used to measure chlorophyll abundance as described in Section 2.2 to calculate the number of remaining phytoplankton cells.

Chapter 3

Results

3.1 ANOVA Analysis

ANOVAs were performed in MATLAB on all calculated individual ingestion rates and total ingestion (the sum of all ingestion rates) for all experiments, relative to groups based on prey concentration. ANOVA was done so that the results from this study could easily be compared to those of previous studies on cannibalism [4, 14]. For experiment with a single cannibalism prey, 2-way ANOVAs were performed with results, and 3-way ANOVAs were performed with results from the experiment with both eggs and nauplii together.

3.1.1 2-Way ANOVA

The ANOVA results for phytoplankton ingestion in presence of eggs are shown in Table 3.1a. Phytoplankton ingestion was primarily affected by phytoplankton concentration followed by egg concentration. The interaction between phytoplankton and egg concentrations also had a significant effect on phytoplankton ingestion. In a two-way interaction, these three parameters explained 82.4% of the variation in phytoplankton ingestion, and phytoplankton concentration alone explained 59.6% of the variation. Figure 3.1d shows phytoplankton ingestion as a function of phytoplankton concentration at nonzero concentrations of eggs, and Figure 3.1c shows phytoplankton ingestion as a function of egg concentration at nonzero concentrations of phytoplankton.

The ANOVA results for egg ingestion are shown in Table 3.1b. Egg ingestion was primarily affected by egg concentration which explained 73.1% of the variation, with no significant interacting effect with phytoplankton concentration. Figure 3.1a shows egg ingestion as a function of egg concentration at nonzero concentrations of phytoplankton.

The ANOVA results for total ingestion of eggs and phytoplankton were similar results to those of phytoplankton ingestion, because significantly more of the total ingestion biomass comes from phytoplankton.

(a)

Parameter	Sum Sq.	d.f.	Mean Sq.	F value	P value	
Egg	10641	3	3547	10.67	0.0001	*
Phyto	55063.6	3	18354.5	55.22	0	*
Egg:Phyto	10391.5	9	1154.6	3.47	0.0045	*
Error	10304.2	31	332.4			
Total	92354.8	46				

(b)

Parameter	Sum Sq.	d.f.	Mean Sq.	F value	P value	
Egg	5.68285	3	1.89428	36.89	0	*
Phyto	0.03604	3	0.01201	0.23	0.872	
Egg:Phyto	0.18302	9	0.02034	0.4	0.9276	
Error	1.59173	31	.05135			
Total	7.7709	46				

Table 3.1: Egg Cannibalism ANOVA Results. *A*: Phytoplankton ingestion results. *B*: Egg ingestion results. Parameters that were significant are denoted with an asterisk.

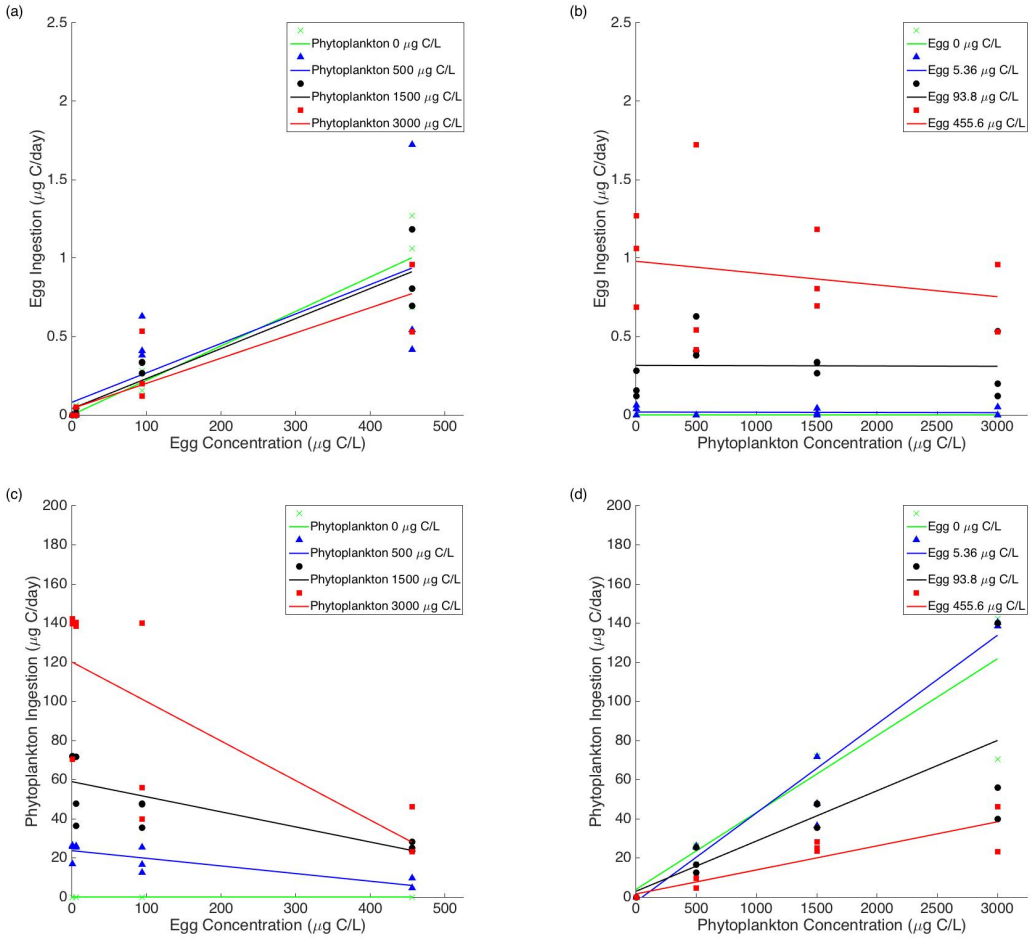


Figure 3.1: Egg Cannibalism ANOVA plots. *A*: Egg ingestion versus egg concentration at various phytoplankton concentrations. *B*: Egg ingestion versus phytoplankton concentration at various egg concentrations. *C*: Phytoplankton ingestion versus egg concentration at various phytoplankton concentrations. *D*: Phytoplankton ingestion versus phytoplankton concentration at various egg concentrations.

The ANOVA results for phytoplankton ingestion in presence of nauplii are shown in Table 3.2a. Phytoplankton ingestion was primarily affected by phytoplankton concentration which explained 97.7% of the variation. Figure 3.2d shows phytoplankton ingestion as a function of phytoplankton concentration at nonzero concentrations of nauplii.

The ANOVA results for nauplii ingestion are shown in Table 3.2b. Nauplii ingestion was primarily affected by nauplii concentration followed by phytoplankton concentration. The interaction between phytoplankton and nauplii concentrations also had a significant effect on nauplii ingestion. In a two-way interaction, these parameters explained 87.4% of the variation in nauplii ingestion, and nauplii concentration alone explained 69.8% of the variation. Figure 3.2a shows nauplii ingestion as a function of nauplii concentration at nonzero concentrations of phytoplankton, and Figure 3.2b shows nauplii ingestion as a function of phytoplankton concentration at nonzero concentrations of nauplii.

As with egg and phytoplankton total ingestion, the ANOVA results for total ingestion of nauplii and phytoplankton were similar results to those of phytoplankton ingestion, because significantly more of the total ingestion biomass comes from phytoplankton.

(a)

Parameter	Sum Sq.	d.f.	Mean Sq.	F value	P value	
Nauplii	35.2	3	11.7	2.28	0.1053	
Phyto	98751.3	3	32917.1	6387.03	0	*
Nauplii:Phyto	95.3	9	10.6	2.06	0.0769	
Error	123.7	24	5.2			
Total	101036.1	39				

(b)

Parameter	Sum Sq.	d.f.	Mean Sq.	F value	P value	
Nauplii	0.04464	3	0.01488	155.44	7.44625×10^{-16}	*
Phyto	0.00492	3	0.00164	17.12	3.68802×10^{-6}	*
Nauplii:Phyto	0.00636	9	0.00071	7.38	4.28188×10^{-5}	*
Error	0.0023	24	0.0001			
Total	0.06394	39				

Table 3.2: Nauplii Cannibalism ANOVA Results. *A*: Phytoplankton ingestion results. *B*: Nauplii ingestion results. Parameters that were significant are denoted with an asterisk.

For single prey cannibalism ANOVA figures in terms of individuals, see Appendix A.1.

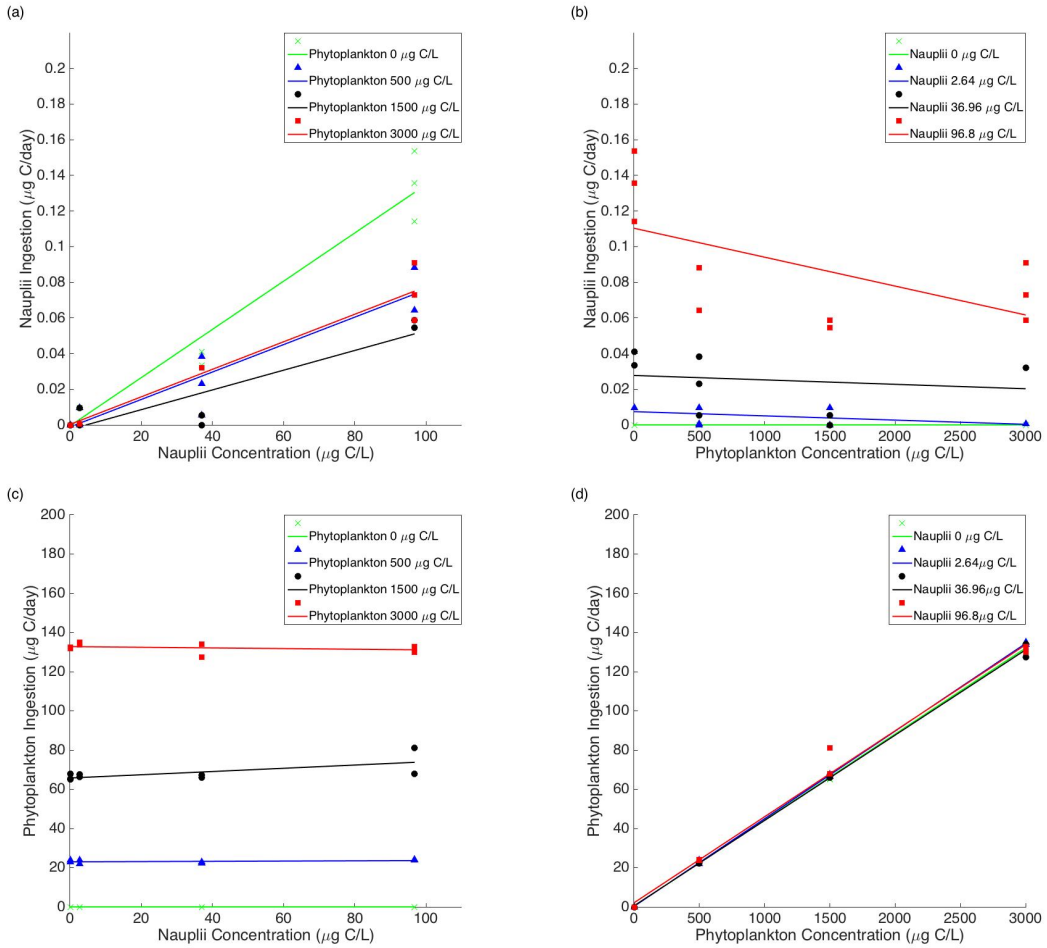


Figure 3.2: Nauplii Cannibalism ANOVA plots. *A*: Nauplii ingestion versus nauplii concentration at various phytoplankton concentrations. *B*: Nauplii ingestion versus phytoplankton concentration at various nauplii concentrations. *C*: Phytoplankton ingestion versus nauplii concentration at various phytoplankton concentrations. *D*: Phytoplankton ingestion versus phytoplankton concentration at various nauplii concentrations.

3.1.2 3-Way ANOVA

The ANOVA results for phytoplankton ingestion are shown in Table 3.3a. Phytoplankton ingestion was primarily affected by phytoplankton concentration which explained 69.8% of the variation. Figure 3.3 shows phytoplankton ingestion as a function of phytoplankton concentration at nonzero concentrations of egg and nauplii.

The ANOVA results for egg ingestion are shown in Table 3.3b. Egg ingestion was primarily affected by egg concentration which explained 81.4% of the variation. Figure 3.4 shows egg ingestion as a function of egg concentration at nonzero concentrations of phytoplankton and nauplii.

The ANOVA results for nauplii ingestion are shown in Table 3.3c. Nauplii ingestion was primarily affected by egg concentration followed by nauplii and then phytoplankton concentration. The interaction between egg and nauplii concentrations also had a significant effect on nauplii ingestion. In a three-way interaction, these parameters explained 90.1% of the variation in nauplii ingestion, and egg concentration alone explained 55.2% of the variation. Figure 3.7 shows nauplii ingestion as a function of nauplii concentration at nonzero concentrations of egg and phytoplankton, Figure 3.5 shows nauplii ingestion as a function of phytoplankton concentration at nonzero concentrations of egg and nauplii, and Figure 3.6 shows nauplii ingestion as a function of egg concentration at nonzero concentrations of phytoplankton and nauplii.

The ANOVA results for total ingestion of phytoplankton, eggs, and nauplii were similar results to those of phytoplankton ingestion, because significantly more of the total ingestion biomass comes from phytoplankton.

For two prey cannibalism ANOVA figures in terms of individuals, see Appendix A.2.

(a) Parameter	Sum Sq.	d.f.	Mean Sq.	F value	P value	
Egg	47.7	3	15.9	2.46	0.0703	
Phyto	1985.56	3	661.854	102.58	0	*
Nauplii	16.68	3	5.561	0.86	0.4656	
Egg:Phyto	94.18	9	10.464	1.62	0.1279	
Egg:Nauplii	47.6	9	5.289	0.82	0.6001	
Phyto:Nauplii	41.23	9	4.581	0.71	0.6976	
Egg:Phyto:Nauplii	198.21	27	7.341	1.14	0.3289	
Error	412.92	64	6.452			
Total	2844.09	127				

(b) Parameter	Sum Sq.	d.f.	Mean Sq.	F value	P value	
Egg	0.00877	3	0.00292	164.89	0	*
Phyto	0.00003	3	0.00001	0.65	0.5886	
Nauplii	0.00003	3	0.00001	0.64	0.5946	
Egg:Phyto	0.00007	9	0.00001	0.44	0.9089	
Egg:Nauplii	0.00011	9	0.00001	0.68	0.7199	
Phyto:Nauplii	0.00014	9	0.00002	0.86	0.5669	
Egg:Phyto:Nauplii	0.00048	27	0.00002	1	0.4877	
Error	0.00161	64	0.00002			
Total	0.01077	127				

(c) Parameter	Sum Sq.	d.f.	Mean Sq.	F value	P value	
Egg	0.09618	3	0.03206	178.47	0	*
Phyto	0.00208	3	0.00069	3.86	0.0133	*
Nauplii	0.02639	3	0.0088	48.98	0	*
Egg:Phyto	0.003	9	0.00033	1.85	0.0756	
Egg:Nauplii	0.03236	9	0.0036	20.02	0	*
Phyto:Nauplii	0.00112	9	0.00012	0.69	0.715	
Egg:Phyto:Nauplii	0.00164	27	0.00006	0.34	0.9987	
Error	0.0115	64	0.00018			
Total	0.17426	127				

Table 3.3: 3-Way ANOVA Results. A: Phytoplankton ingestion results. B: Egg ingestion results. C: Nauplii ingestion results. Parameters that were significant are denoted with an asterisk.

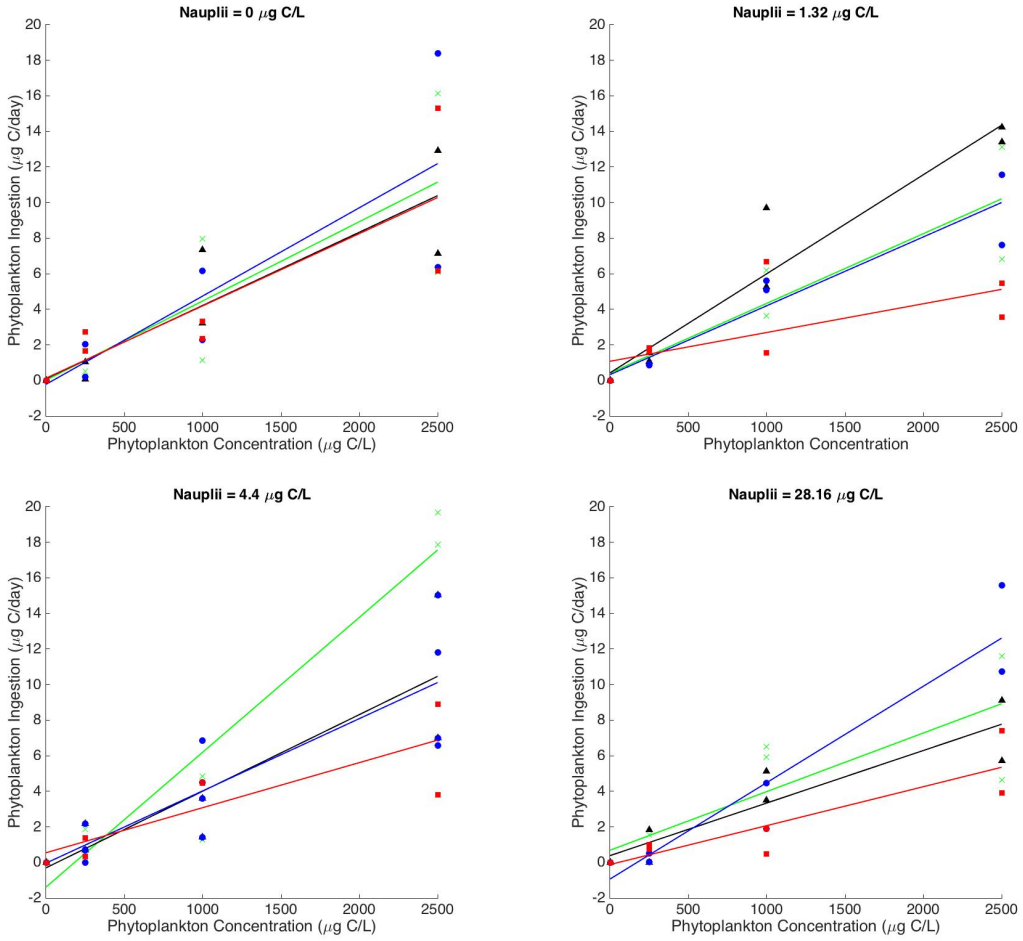


Figure 3.3: Phytoplankton ingestion versus phytoplankton concentration at various concentrations of egg and nauplii. The green line and data points show phytoplankton ingestion with egg concentration of 0 μg C/L. The black line and data points show phytoplankton ingestion with egg concentration of 2.68 μg C/L. The blue line and data points show phytoplankton ingestion with egg concentration of 53.6 μg C/L. The red line and data points show phytoplankton ingestion with egg concentration of 187.6 μg C/L.

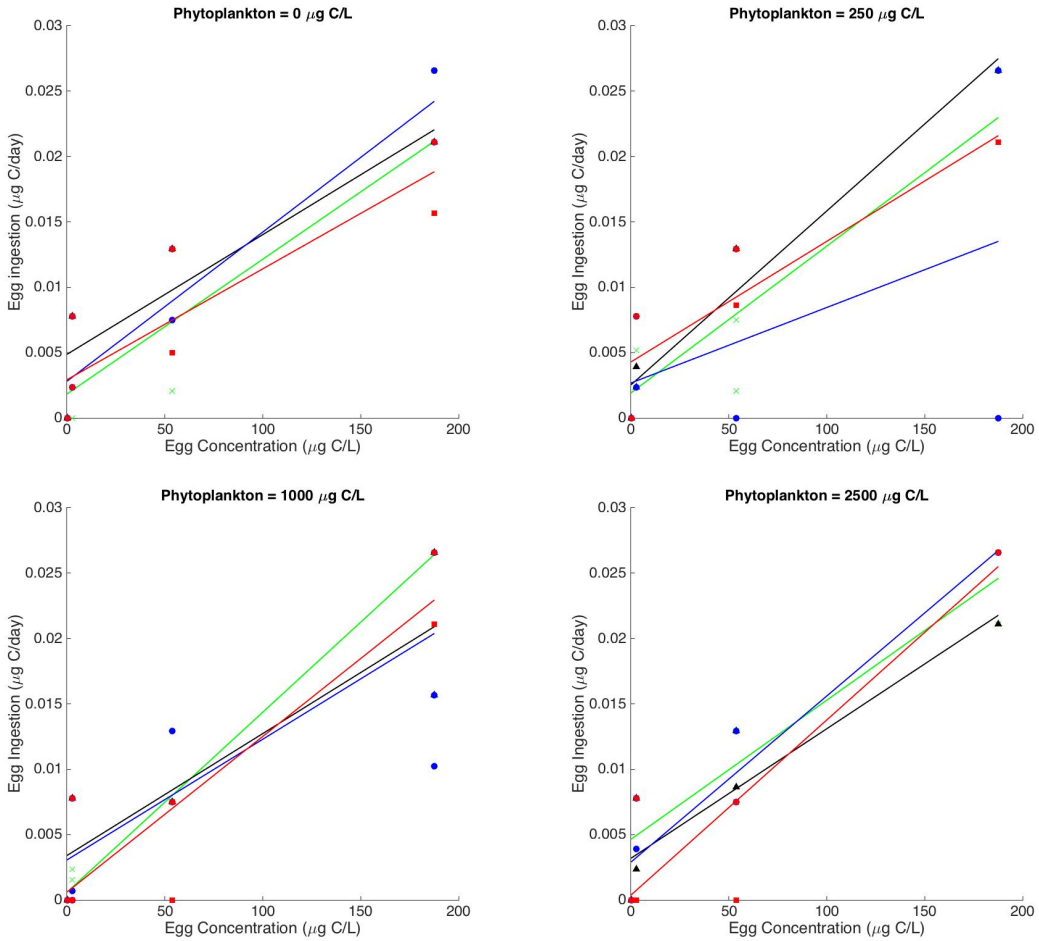


Figure 3.4: Egg ingestion versus egg concentration at various concentrations of phytoplankton and nauplii. The green line and data points show egg ingestion with nauplii concentration of 0 μg C/L. The black line and data points show egg ingestion with nauplii concentration of 1.32 μg C/L. The blue line and data points show egg ingestion with nauplii concentration of 4.4 μg C/L. The red line and data points show egg ingestion with nauplii concentration of 28.16 μg C/L.

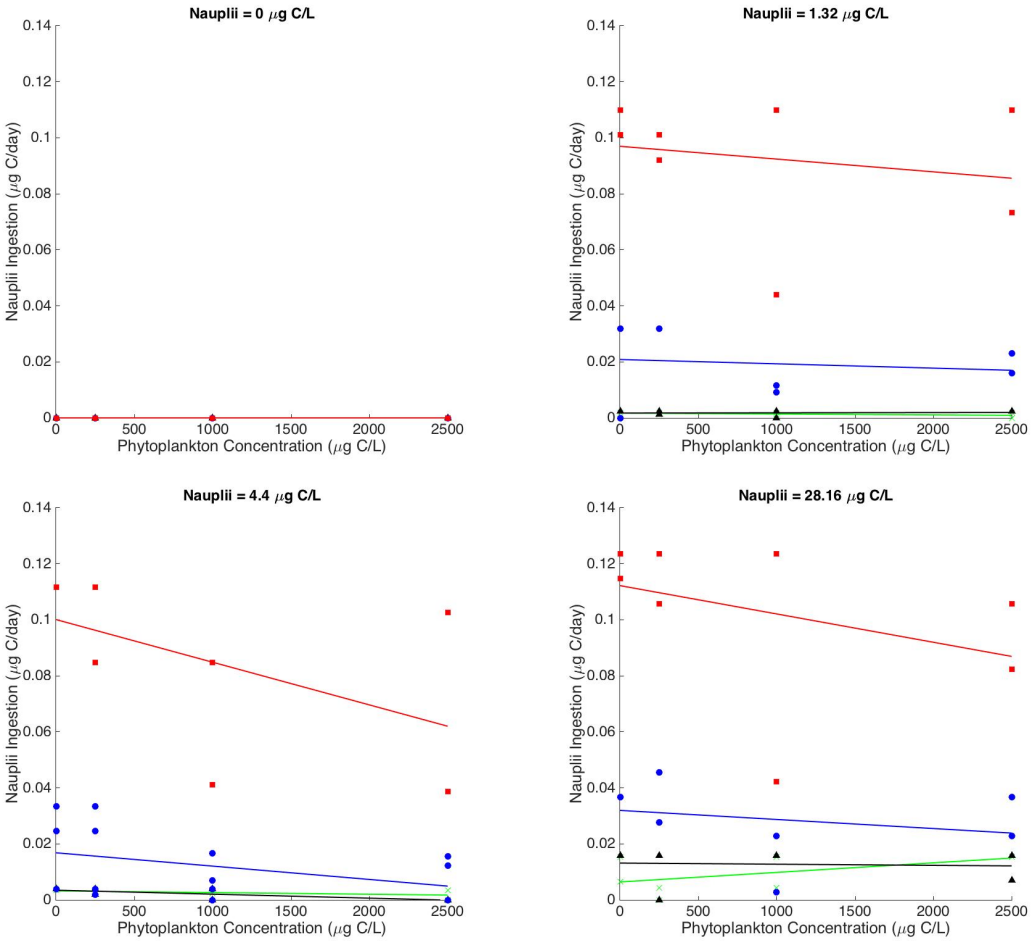


Figure 3.5: Nauplii ingestion versus phytoplankton concentration at various concentrations of eggs and nauplii. The green line and data points show nauplii ingestion with egg concentration of 0 μg C/L. The black line and data points show nauplii ingestion with egg concentration of 2.68 μg C/L. The blue line and data points show nauplii ingestion with egg concentration of 53.6 μg C/L. The red line and data points show nauplii ingestion with egg concentration of 187.6 μg C/L.

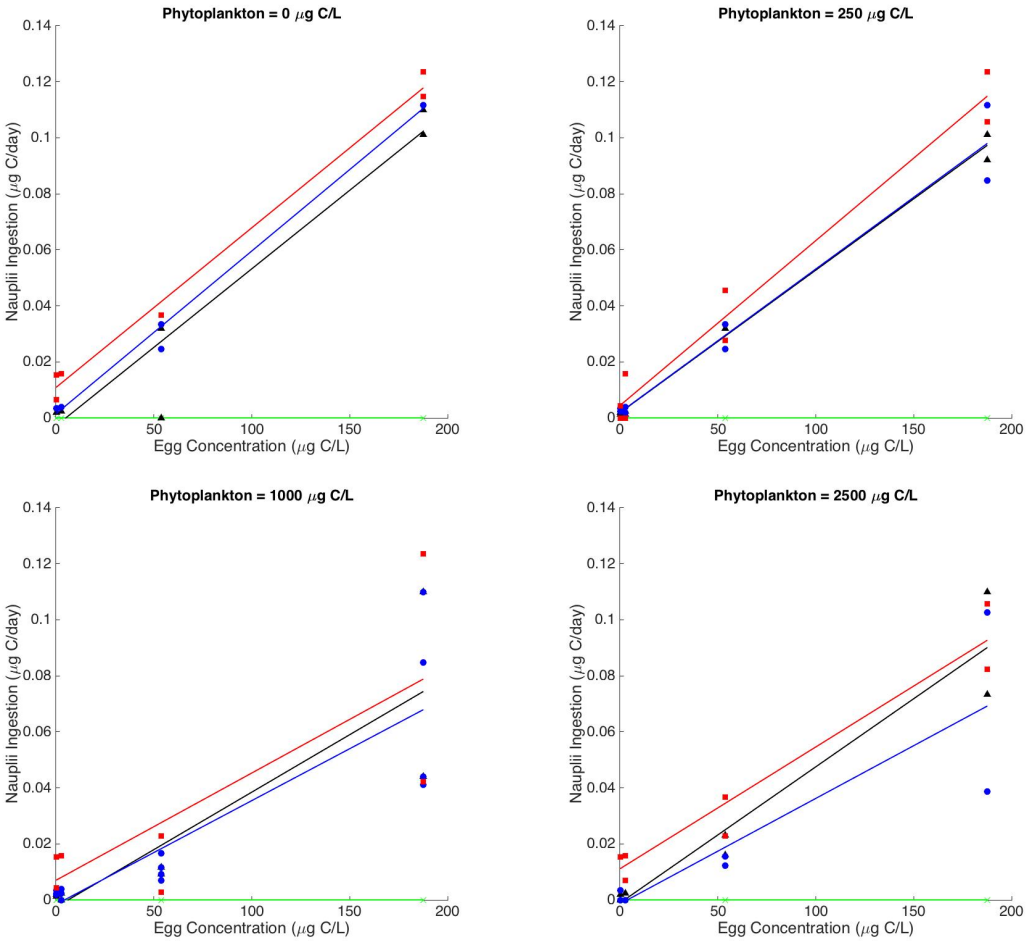


Figure 3.6: Nauplii ingestion versus egg concentration at various concentrations of phytoplankton and nauplii. The green line and data points show nauplii ingestion with nauplii concentration of 0 μg C/L. The black line and data points show nauplii ingestion with nauplii concentration of 1.32 μg C/L. The blue line and data points show nauplii ingestion with nauplii concentration of 4.4 μg C/L. The red line and data points show nauplii ingestion with nauplii concentration of 28.16 μg C/L.

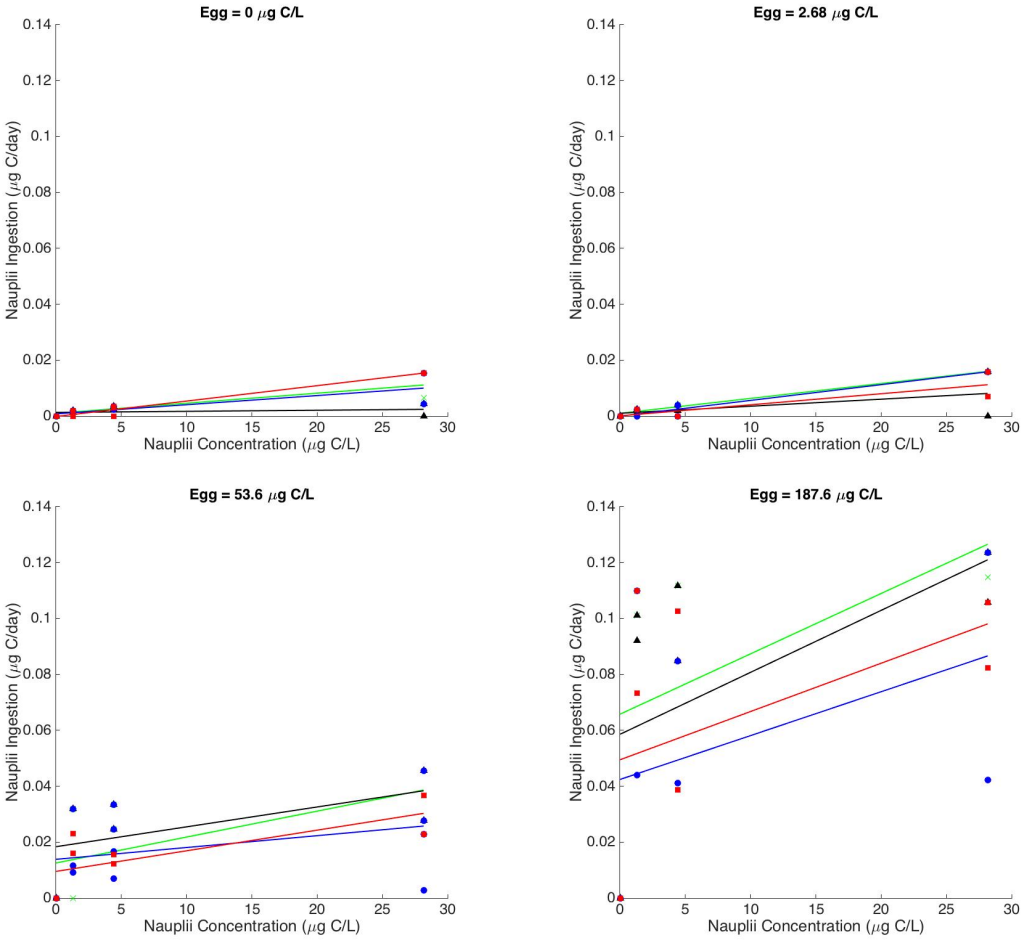


Figure 3.7: Nauplii ingestion versus nauplii concentration at various concentrations of phytoplankton and eggs. The green line and data points show nauplii ingestion with phytoplankton concentration of 0 μg C/L.The black line and data points show nauplii ingestion with phytoplankton concentration of 250 μg C/L. The blue line and data points show nauplii ingestion with phytoplankton concentration of 1000 μg C/L. The red line and data points show nauplii ingestion with phytoplankton concentration of 2500 μg C/L.

3.2 Ingestion Fits

For each individual measured ingestion rate and calculated total ingestion from all cannibalism experiments, the data was used to fit a model of the ingestion rate in MATLAB. The ingestion rates were fit with nonlinear models, as this is what is typically used in NPZ models and the ANOVAs are analogous to performing a linear model fit. Thus, model selection can be performed to determine if ingestion and cannibalism is best described in linear or nonlinear terms.

The functional form for the ingestion fits is Holling Type III. This functional form was chosen, as it is traditionally used to describe feeding behavior of generalist predators, or predators with more than one food source. This functional form has a sigmoidal shape with a depressed response of predators at low prey densities to take into account switching behavior. The maximum grazing rate is represented by g, and prey choice is represented by the c parameters.

3.2.1 Single Cannibalism Prey

The following equations were used to fit phytoplankton ingestion (3.1) and egg ingestion (3.2) in the presence of both phytoplankton and eggs:

$$PI_{egg} = \frac{g^{PI} \cdot c_p^{PI} P^2}{1 + c_p^{PI} P^2 + c_e^{PI} E^2} \tag{3.1}$$

$$EI_{egg} = \frac{g^{EI} \cdot c_e^{EI} E^2}{1 + c_p^{EI} P^2 + c_e^{EI} E^2}. \tag{3.2}$$

Table 3.4a shows the best fit parameter values for phytoplankton ingestion, with $R^2 = 0.821$. The fitted phytoplankton ingestion model is shown in Figure 3.8a with the actual phytoplankton ingestion data and means at each initial concentration combination. Table 3.4b shows the best fit parameter values for egg ingestion, with $R^2 = 0.77$. The fitted egg ingestion model is shown in Figure 3.8b with the actual egg ingestion data and means at each initial concentration combination.

The following equation was used to fit total ingestion of eggs and phytoplankton:

$$TI_{egg} = \frac{g \cdot (c_p P^2 + c_e E^2)}{1 + c_p P^2 + c_e E^2}. \tag{3.3}$$

Table 3.5 shows the best fit parameter values, with $R^2 = 0.668$. The fitted model is shown in Figure 3.9 with the actual sums of egg and phytoplankton ingestion data and means at each initial concentration combination.

In addition to the total ingestion fit, the individual egg and phytoplankton ingestion rate fits were summed to see how well they explained calculated total

(a) Parameter	Value	P Value
g^{PI}	168.81	0
c_p^{PI}	2.9633×10^{-7}	1.477×10^{-8}
c_e^{PI}	0.00012631	0.032535

(b) Parameter	Value	P Value
g^{EI}	0.98164	8.1214×10^{-16}
c_p^{EI}	6.4533×10^{-8}	0.59827
c_e^{EI}	6.0181×10^{-5}	0.014953

Table 3.4: Egg Cannibalism Individual Ingestion Fits Parameter Values. A: Phytoplankton ingestion fit parameter values. B: Egg ingestion fit parameter values.

ingestion rates. The summed total ingestion had $R^2 = 0.8235$, which is considerably higher than that of the total ingestion fit.

Parameter	Value	P Value
g	138.9	0
c_p	2.6455×10^{-7}	7.5783×10^{-7}
c_e	-2.2575×10^{-7}	0.52089

Table 3.5: Egg Cannibalism: total ingestion fit parameter values.

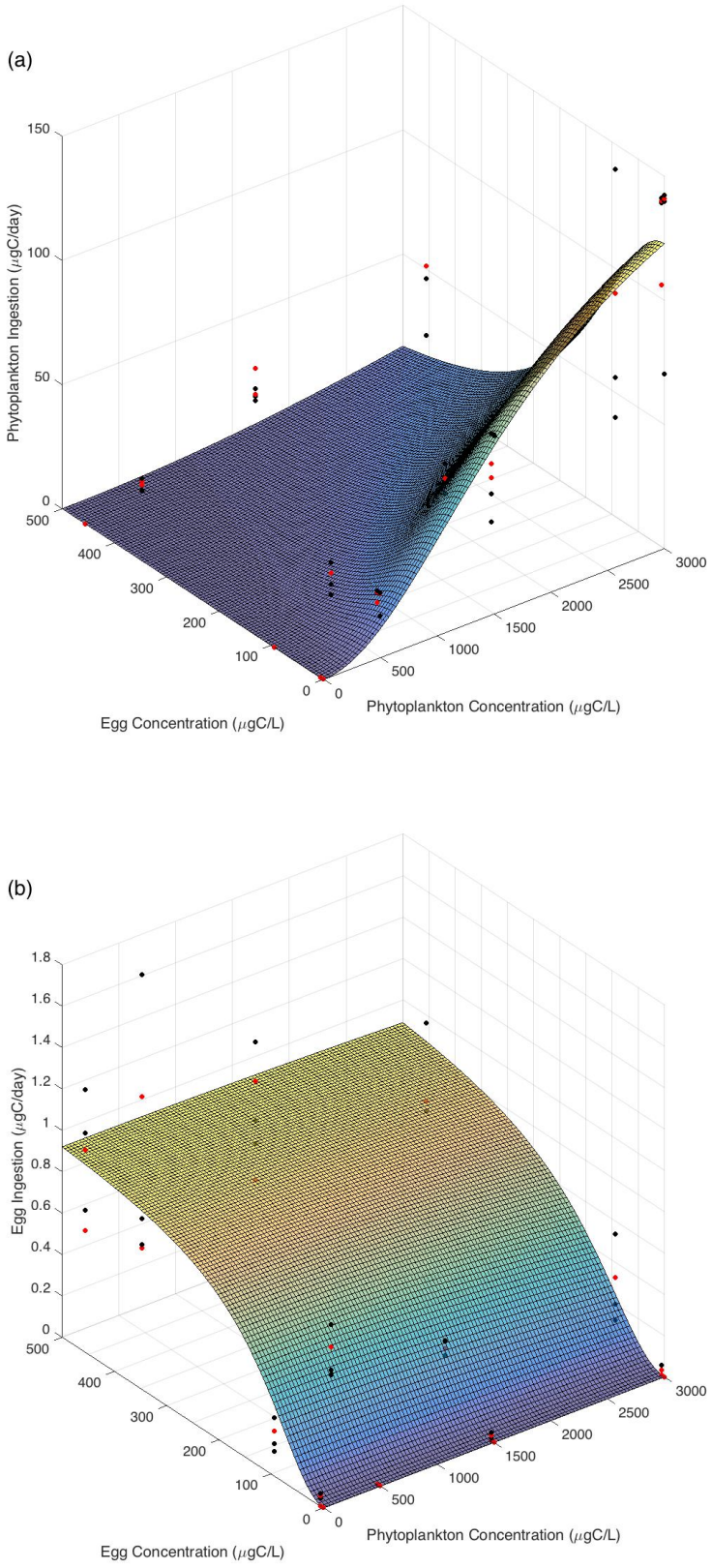


Figure 3.8: Egg Cannibalism Individual Ingestion Fits. The black closed circles are actual data points. The red closed circles are the means of the measured ingestion rates. A: Phytoplankton ingestion fit. B: Egg ingestion fit.

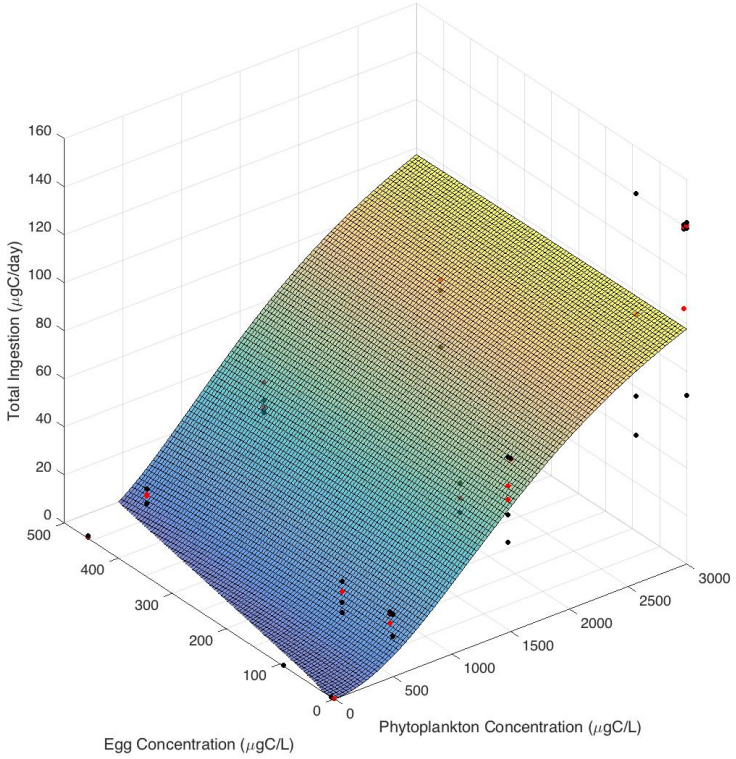


Figure 3.9: Egg Cannibalism: total ingestion. The black closed circles are actual data points. The red closed circles are the means of the measured ingestion rates.

The following equations were used to fit phytoplankton ingestion (3.4) and nauplii ingestion (3.5) in the presence of both phytoplankton and nauplii:

$$PI_{nauplii} = \frac{g^{PI} \cdot c_p^{PI} P^2}{1 + c_p^{PI} P^2 + c_n^{PI} N^2} \tag{3.4}$$

$$NI_{nauplii} = \frac{g^{NI} \cdot c_n^{NI} N^2}{1 + c_p^{NI} P^2 + c_n^{NI} N^2}. \tag{3.5}$$

Table 3.6a shows the best fit parameter values for phytoplankton ingestion, with $R^2 = 0.985$. The fitted phytoplankton ingestion model is shown in Figure 3.10a with the actual phytoplankton ingestion data and means at each initial concentration combination. Table 3.6b shows the best fit parameter values for nauplii ingestion, with $R^2 = 0.826$. The fitted nauplii ingestion model is shown in Figure 3.10b with the actual nauplii ingestion data and means at each initial concentration combination.

(a)

Parameter	Value	P Value
g^{PI}	178	0
c_p^{PI}	2.9774×10^{-7}	1.9593×10^{-23}
c_n^{PI}	-1.0023×10^{-5}	0.24203

(b)

Parameter	Value	P Value
g^{NI}	0.19365	0.0028623
c_p^{NI}	1.6864×10^{-7}	0.064039
c_n^{NI}	0.00012726	0.12094

Table 3.6: Nauplii Cannibalism Individual Ingestion Fits Parameter Values. *A*: Phytoplankton ingestion fit parameter values. *B*: Nauplii ingestion fit parameter values.

The following equation was used to fit total ingestion of nauplii and phytoplankton:

$$TI_{nauplii} = \frac{g \cdot (c_p P^2 + c_n N^2)}{1 + c_p P^2 + c_n N^2}. \tag{3.6}$$

Table 3.7 shows the best fit parameter values, with $R^2 = 0.986$. The fitted model is shown in Figure 3.9 with the actual sums nauplii and phytoplankton ingestion data and means at each initial concentration combination.

In addition to the total ingestion fit, the individual nauplii and phytoplankton ingestion rate fits were summed to see how well they explained calculated total ingestion rates. The summed total ingestion had $R^2 = 0.9856$, which is very close to that of the total ingestion fit.

Parameter	Value	P Value
g	181.42	0
c_p	2.8849×10^{-7}	7.4326×10^{-27}
c_n	3.3388×10^{-6}	0.062452

Table 3.7: Nauplii Cannibalism: total ingestion fit parameter values.

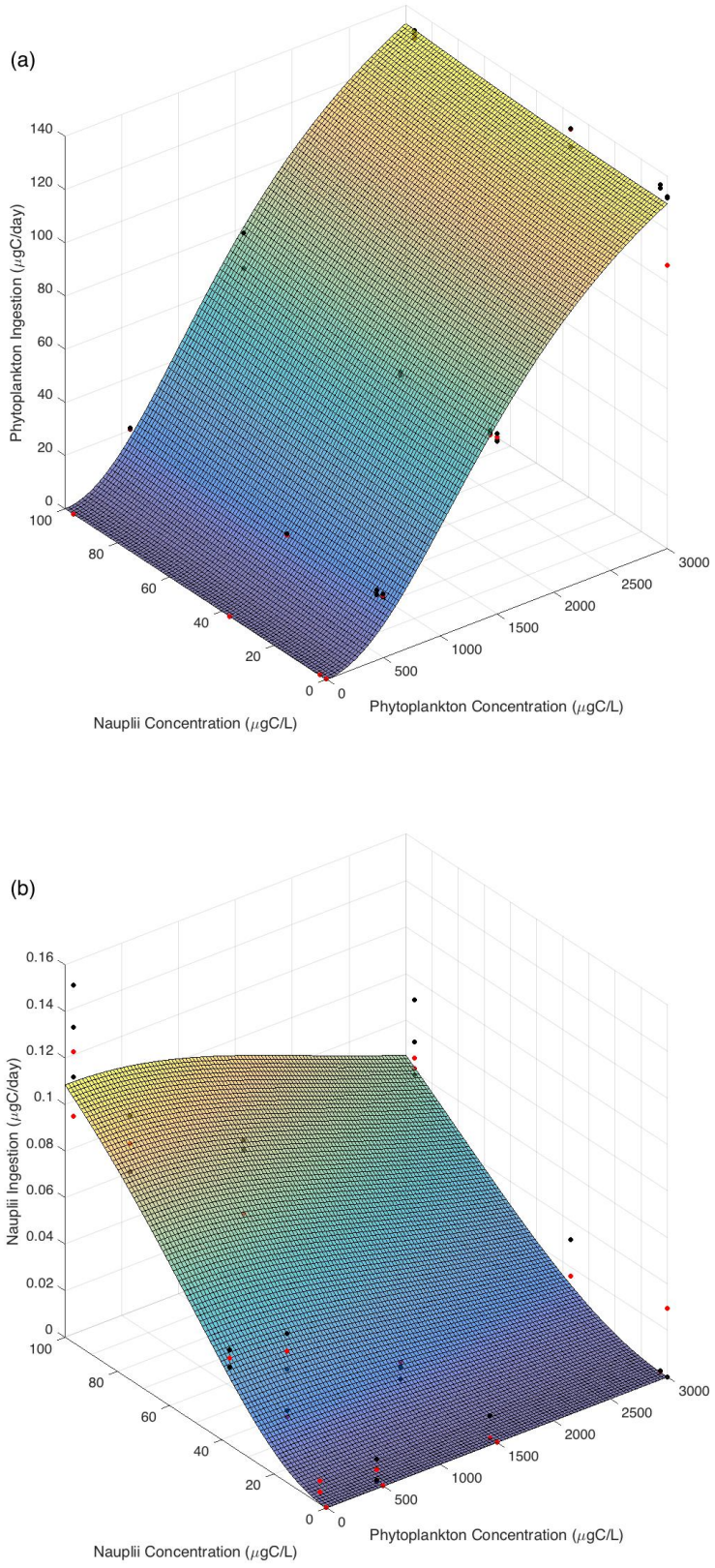


Figure 3.10: Nauplii Cannibalism Individual Ingestion Fits. The black closed circles are actual data points. The red closed circles are the means of the measured ingestion rates. A: Phytoplankton ingestion fit. B: Nauplii ingestion fit.

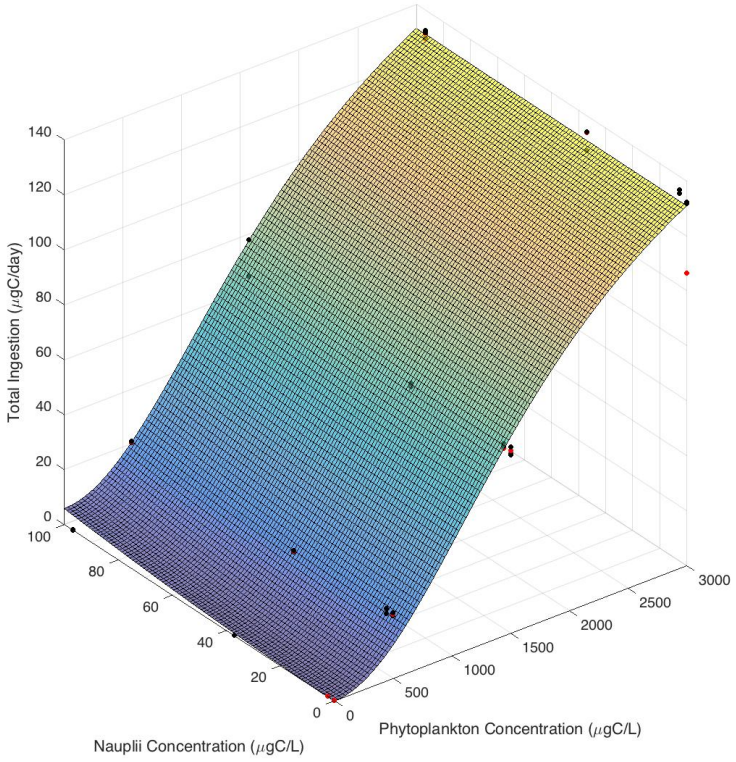


Figure 3.11: Nauplii Cannibalism: total ingestion. The black closed circles are actual data points. The red closed circles are the means of the measured ingestion rates.

3.2.2 Two Cannibalism Prey

The following equations were used to fit phytoplankton ingestion in the presence of eggs and nauplii (3.7), egg ingestion in the presence of phytoplankton and nauplii (3.9), and nauplii ingestion in the presence of phytoplankton and eggs (3.9):

$$PI = \frac{g^{PI} \cdot c_p^{PI} P^2}{1 + c_p^{PI} P^2 + c_e^{PI} E^2 + c_n^{PI} N^2} \tag{3.7}$$

$$EI = \frac{g^{EI} \cdot c_e^{EI} E^2}{1 + c_p^{EI} P^2 + c_e^{EI} E^2 + c_n^{EI} N^2} \tag{3.8}$$

$$NI = \frac{g^{NI} \cdot c_n^{NI} N^2}{1 + c_p^{NI} P^2 + c_e^{NI} E^2 + c_n^{NI} N^2}. \tag{3.9}$$

Table 3.8a shows the parameter values for phytoplankton ingestion calculated to have the best fit with, $R^2 = 0.739$. Table 3.8b shows the parameter values for egg ingestion calculated to have the best fit with, $R^2 = 0.746$. Table 3.8c shows the parameter values for nauplii ingestion calculated to have the best fit with, $R^2 = 0.838$.

(a) Parameter	Value	P Value
g^{PI}	15.0982	1.915×10^{-19}
c_p^{PI}	5.4285×10^{-7}	0.00056534
c_e^{PI}	6.4029×10^{-5}	0.012374
c_n^{PI}	0.0008185	0.15139

(b) Parameter	Value	P Value
g^{EI}	0.025794	1.0692×10^{-38}
c_p^{EI}	-4.9083×10^{-8}	0.17701
c_e^{EI}	0.00018608	1.9803×10^{-5}
c_n^{EI}	0.00058026	0.26004

(c) Parameter	Value	P Value
g^{NI}	0.094868	0
c_p^{NI}	8.0285×10^{-12}	0.51989
c_e^{NI}	-2.8414×10^{-4}	0
c_n^{NI}	0.00027956	7.3975×10^{-6}

Table 3.8: Two Cannibalism Prey Individual Ingestion Fits Parameter Values. A: Phytoplankton ingestion fit parameter values. B: Egg ingestion fit parameter values. C: Nauplii ingestion fit parameter values.

The following equation was used to fit total ingestion of phytoplankton, eggs,

and nauplii:

$$TI = \frac{g \cdot (c_p P^2 + c_e E^2 + c_n N^2)}{1 + c_p P^2 + c_e E^2 + c_n N^2}. \tag{3.10}$$

Table 3.9 shows the parameter values calculated to have the best fit with, $R^2 = 0.694$.

In addition to the total ingestion fit, the individual egg, nauplii, and phytoplankton ingestion rate fits were summed to see how well they explained calculated total ingestion rates. The summed total ingestion had $R^2 = 0.7406$, which is slightly higher than that of the total ingestion fit.

Parameter	Value	P Value
g	13.343	5.8948×10^{-19}
c_p	5.0138×10^{-7}	0.00043437
c_e	4.9022×10^{-7}	.7426
c_n	-5.4046×10^{-6}	0.93335

Table 3.9: Two Cannibalism Prey Total Ingestion Fit Parameter Values

Chapter 4

Discussion

Based on the 2-way ANOVA on ingestion rates from single cannibalism prey experiments, there was a significant positive relationship between egg cannibalism and egg concentration (see Figure 3.1a and Table 3.1b). However, unlike in a previous study [4], there was not a significant relationship between egg ingestion and phytoplankton concentration (see Figure 3.1b). This may be because the concentrations of eggs used in this experiment were much greater than that of the previous study, but even in the two cannibalism prey experiment which had egg concentrations closer to that of the previous study, a significant relationship between egg ingestion and phytoplankton concentration was not evident based on the 3-way ANOVA (see Table 3.3b).

A significant positive relationship between nauplii ingestion and nauplii concentration and a significant negative relationship between nauplii ingestion and phytoplankton concentration were evident from the 2-way ANOVA (see Table 3.2b, Figure 3.2a, and Figure 3.2b). This is consistent with results from a previous study [14]. The 3-way ANOVA confirmed these results, also showing a significant positive relationship between nauplii ingestion and nauplii concentration and a significant negative relationship between nauplii ingestion and phytoplankton concentration (see Table 3.3c, Figure 3.7, and Figure 3.5). Interestingly, there also appears to be a significant positive relationship between nauplii ingestion and egg concentration, in addition to a significant interaction between egg and nauplii concentrations (see Figure 3.6 and Table 3.3c).

The egg cannibalism 2-way ANOVA showed a significant positive relationship between phytoplankton ingestion and phytoplankton concentration and a significant negative relationship between phytoplankton ingestion and egg concentration with the interaction between phytoplankton and egg concentrations also being significant (see Table 3.1a, Figure 3.1d, and Figure 3.1c). However, there was only a significant positive relationship between phytoplankton ingestion and phytoplank-

ton concentration in nauplii cannibalism 2-way and in 3-way ANOVA (see Table 3.2a, Figure 3.2d, and Table 3.3a).

The Holling Type III functional response produced reasonably good fits for all the individual ingestion rates with the best fits resulting from data from the experiment with nauplii as the only cannibalism prey (see Figure 3.10a and Figure 3.10b) and from nauplii ingestion data from the two cannibalism prey experiment. Within the nauplii ingestion fit for the two cannibalism prey experiment, the c_e^{NI} parameter was significantly negative (see Table 3.8). All other negative coefficients from the nonlinear fits were not significant from zero. The negative value for c_e^{NI} validates the linear plots from the ANOVA results which show nauplii ingestion increasing with increasing egg concentration. This unitinuitive result may be due to error in counting methods when measuring ingestion rates or some yet unknown interaction. Because the two prey cannibalism experiment only had two replicates, repeated experiments are necessary to elucidate the true relationship between nauplii ingestion and egg concentration.

The total ingestion fits had the lowest R^2 values. More work is necessary to determine if Holling Type III or some other functional response, like the one proposed in a previous study [16], is the best at describing cannibalistic behavior in *A. tonsa*. Also, in this study, the ingestion rates were all fit separately, but multivariate regression might be another option worth exploring, in particular to arrive at a model with fewer parameters. Model selection to determine models of best fit, for example using AIC, is a natural next step, comparing in particular linear models to Holling Type functional responses.

While this study provides valuable insight into quantifying cannibalistic behavior and related ingestion rates in *A. tonsa*, further data collection on ingestion rates with increased range and resolution of egg and nauplii concentrations would help improve the accuracy of cannibalism models. In particular, several times there were nonsignificant P-values, specifically in the c coefficients, and these parameters seemed to have high uncertainty.

References

[1] U. Berggreen, B. Hansen, and T. Kiørboe. Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. *Marine Biology*, 99:341–352, 1988.

[2] Maarten Boersma, Anne Wesche, and Hans-Jürgen Hirche. Predation of calanoid copepods on their own and other copepods offspring. *Marine Biology*, 161:733–743, April 2014.

[3] Mark Brush. Personal communication. Gave biomass values for *Acartia tonsa* currently used by Chesapeake Bay Program. 3 September 2014.

[4] Guillaume Drillet, Rémi Maguet, Mohamed-Sofiane Mahjoub, Francois Roullier, and Mark J. Fielding. Egg cannibalism in *Acartia tonsa*: effects of stocking density, algal concentration, and egg availability. *Aquacult International*, 22:1295–1306, June 2014.

[5] David T. Elliott and Kam W. Tang. The influence of carcass abundance on estimates of mortality and assessment of population dynamics in *Acartia tonsa*. *Marine Ecology Progress Series*, 427:1–12, April 2011.

[6] Michael B. Finiguerra, Hans G. Dam, David E. Avery, and Zair Burris. Sex-specific tolerance to starvation in the copepod *Acartia tonsa*. *Journal of Experimental Marine Biology and Ecology*, 446:17–21, May 2013.

[7] Ray Philip Gerber. *An identification manual to the coastal and estuarine zooplankton of the Gulf of Maine region from Passamaquoddy Bay to Long Island Sound, Part 2*. Acadia Productions, 2000.

[8] Adrianna Ianora. Copepod life history traits in subtemperate regions. *Journal of Marine Systems*, 15:337–349, 1998.

[9] S. W. Jeffery and G. F. Humphrey. New spectrophotometric equations for determining chlorophylls a1, b1, c1, and c2 in higher plants, algae, and natural phytoplankton. *Biochem. Physiol. Pflanz*, 167:191–194, 1975.

[10] Ruth H. Jones, Kevin J. Flynn, and Thomas R. Anderson. Effect of food quality on carbon and nitrogen growth efficiency in the copepod *Acartia tonsa*. *Marine Ecology Progress Series*, 235:147–156, June 2002.

[11] Catherine King, Katherine Shipman, Sarah Day, and Drew LaMar. Dimension and mortality in linear stage class models of *Acartia tonsa*. *Proceedings of the Sixth Symposium on Biomathematics and Ecology: Education and Research*, 2013.

[12] Thomas Kiørboe, Enric Saiz, and Markku Viitasalo. Prey switching behaviour in the planktonic copepod *Acartia tonsa*. *Marine Ecology Progress Series*, 143:64–75, November 1996.

[13] Sérgio M. Leandro, Pete Tiselius, and Henrique Queiroga. Growth and Development of nauplii and copepodites of the estuarine copepod *Acartia tonsa* from southern Europe (Ria de Aveiro, Portugal) under saturating food conditions. *Marine Biology*, 150:121–129, May 2006.

[14] Jason T. Lemus. *The effect of copepod density of cannibalism, survival, development rate, and egg production and the implications for population growth rate and demographics of Acartia tonsa Dana (Copepoda:Calanoida).* PhD thesis, University of Southern Mississippi, December 2006.

[15] Aditee Mitra. Are closure terms appropriate or necessary descriptors of zooplankton loss in nutrient-phytoplankton-zooplankton type models?. *Ecological Modeling*, 220:611–620, January 2009.

[16] Andrew Morozov and Sergei Petrovskii. Feeding on multiple sources: towards a universal parameterization of the functional response of a generalist predator allowing for switching. *PLOS One*, 8:1–9, September 2013.

[17] Kam W. Tang and Maiyai Taal. Trophic modification of food quality by heterotrophic protists: species-specific effects on copepod egg production and egg hatching. *Journal of Experimental Marine Biology and Ecology*, 318:85–98, 2005.

Appendix A

Additional ANOVA Figures

A.1 2-Way ANOVA

This section include additional figures showing results from ANOVAs for the single prey cannibalism experiments. Figures A.1 and A.2 are constructed in the same way as those in Section 3.1.1, but the units are in terms of individuals or cell (phytoplankton) per liter.

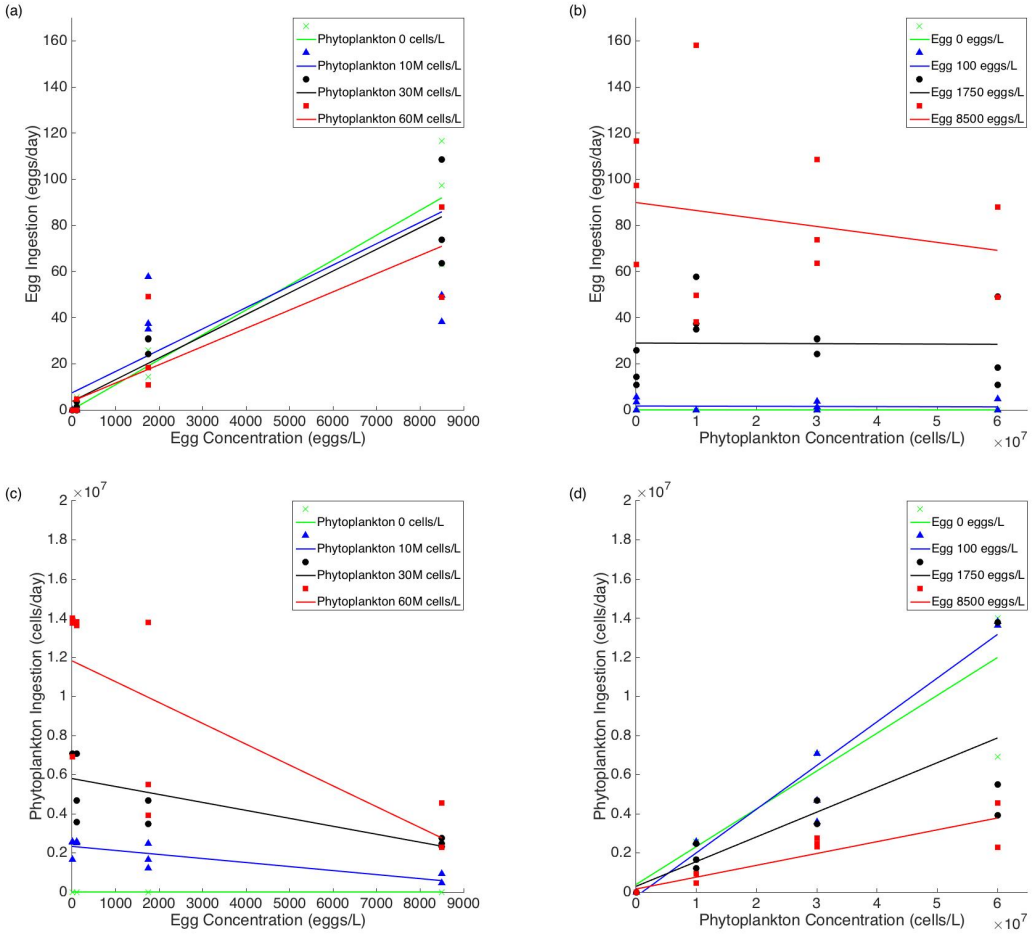


Figure A.1: Egg Cannibalism ANOVA plots. *A*: Egg ingestion versus egg concentration at various phytoplankton concentrations. *B*: Egg ingestion versus phytoplankton concentration at various egg concentrations. *C*: Phytoplankton ingestion versus egg concentration at various phytoplankton concentrations. *D*: Phytoplankton ingestion versus phytoplankton concentration at various egg concentrations.

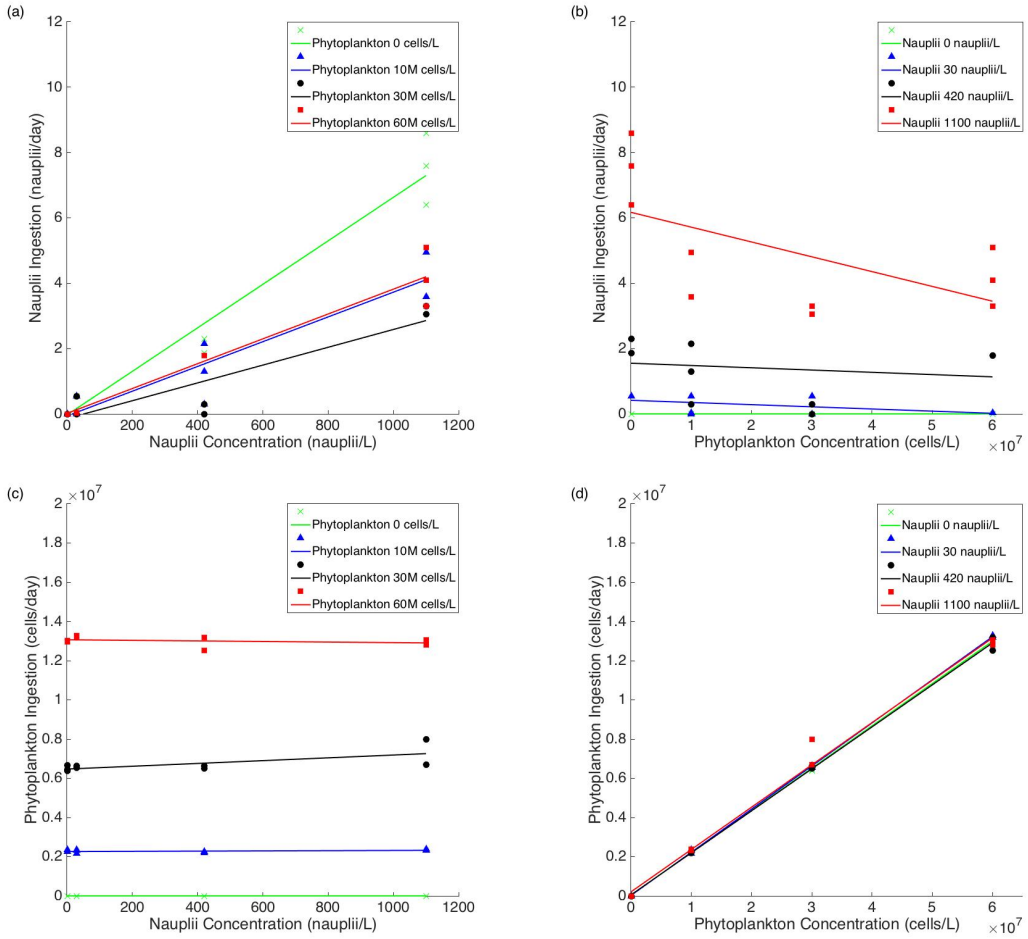


Figure A.2: Nauplii Cannibalism ANOVA plots. *A*: Nauplii ingestion versus nauplii concentration at various phytoplankton concentrations. *B*: Nauplii ingestion versus phytoplankton concentration at various nauplii concentrations. *C*: Phytoplankton ingestion versus nauplii concentration at various phytoplankton concentrations. *D*: Phytoplankton ingestion versus phytoplankton concentration at various nauplii concentrations.

A.2 3-Way ANOVA

This section include additional figures showing results from ANOVAs for the two prey cannibalism experiments. Figures A.3, A.4, A.5, A.6, and A.7 are constructed in the same way as those in Section 3.1.2, but the units are in terms of individuals or cell (phytoplankton) per liter.

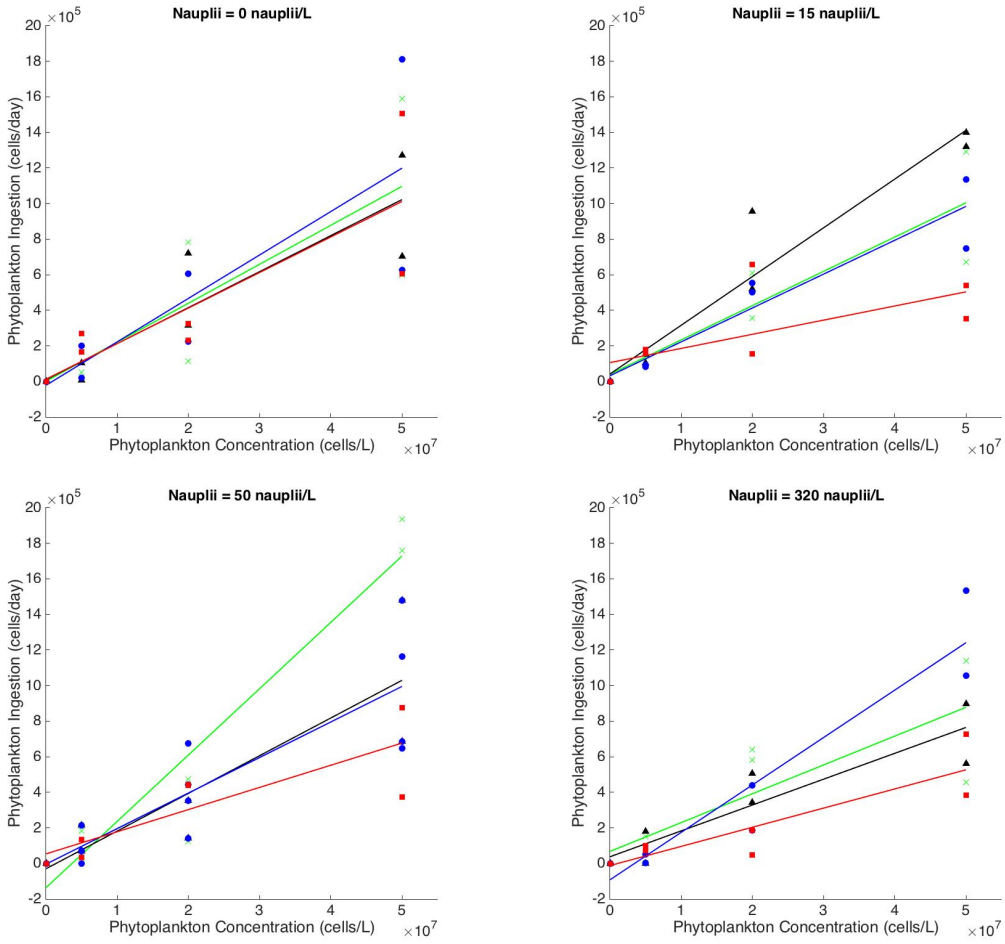


Figure A.3: Phytoplankton ingestion versus phytoplankton concentration at various concentrations of egg and nauplii. The green line and data points show phytoplankton ingestion with egg concentration of 0 eggs/L. The black line and data points show phytoplankton ingestion with egg concentration of 50 eggs/L. The blue line and data points show phytoplankton ingestion with egg concentration of 1000 eggs/L. The red line and data points show phytoplankton ingestion with egg concentration of 3500 eggs/L.

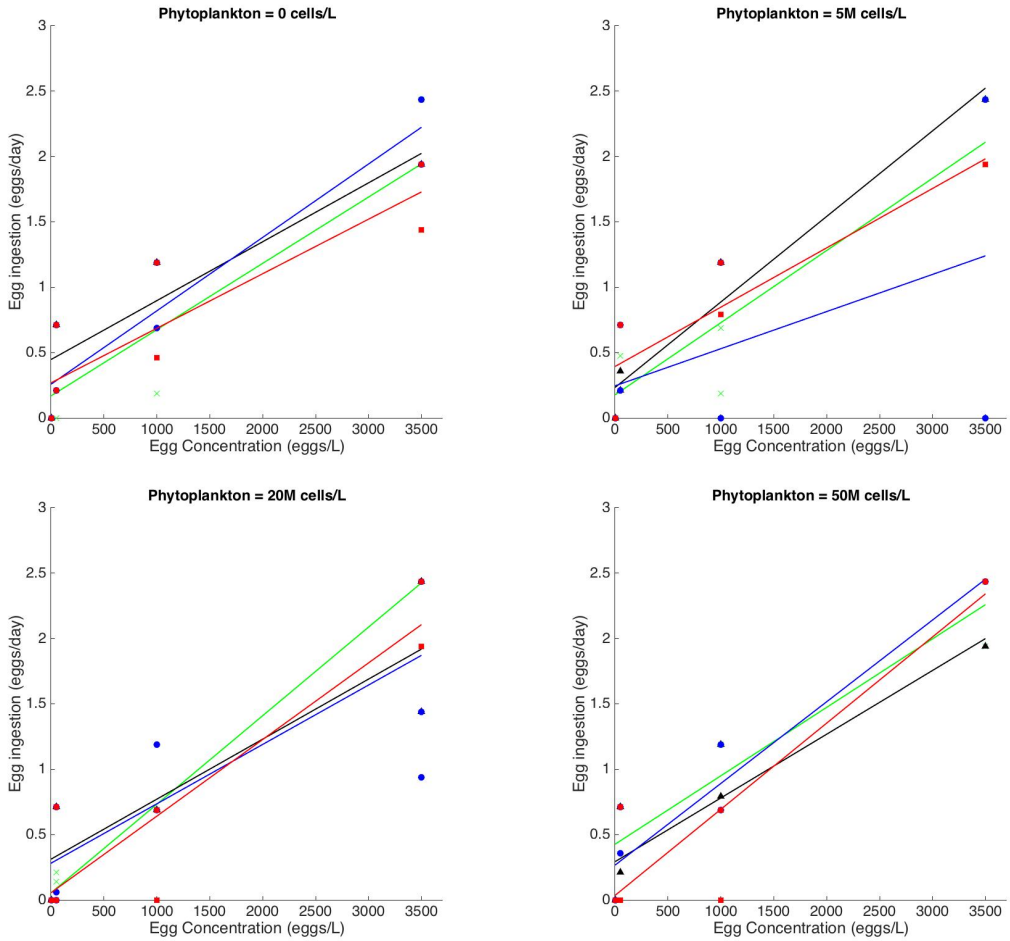


Figure A.4: Egg ingestion versus egg concentration at various concentrations of phytoplankton and nauplii. The green line and data points show egg ingestion with nauplii concentration of 0 nauplii/L. The black line and data points show egg ingestion with nauplii concentration of 15 nauplii/L. The blue line and data points show egg ingestion with nauplii concentration of 50 nauplii/L. The red line and data points show egg ingestion with nauplii concentration of 320 nauplii/L.

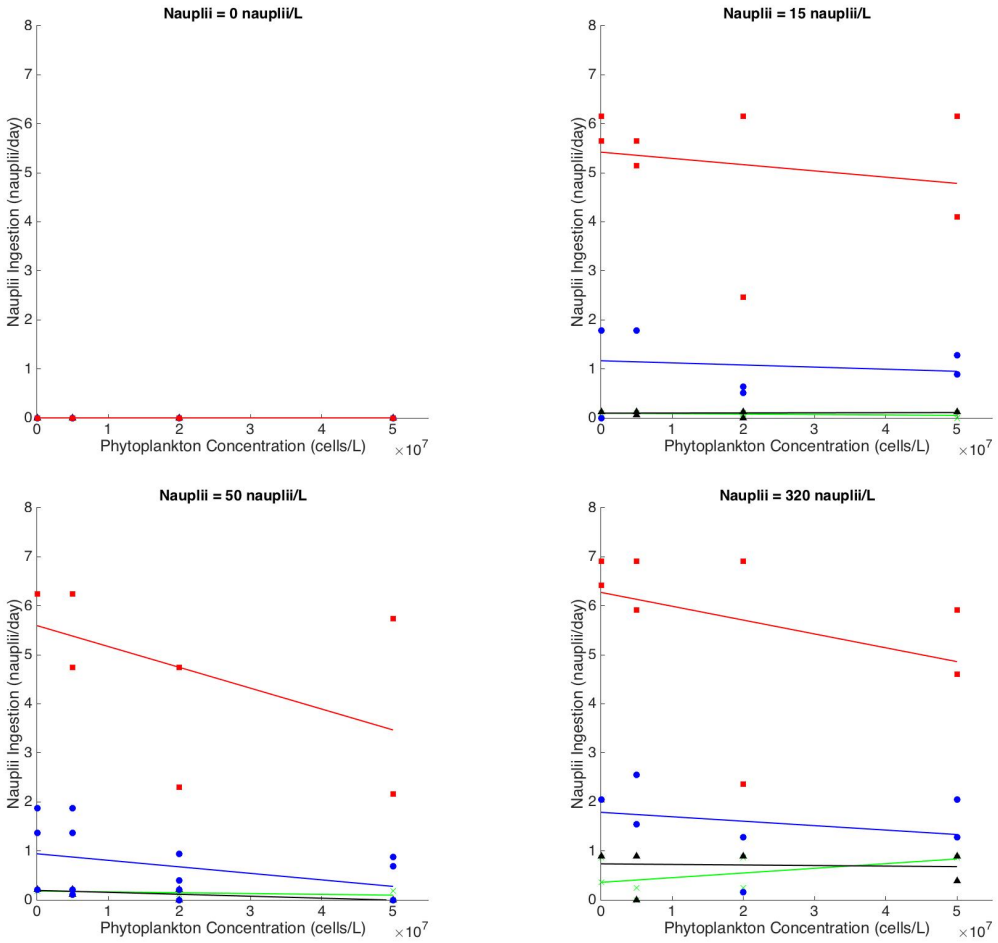


Figure A.5: Nauplii ingestion versus phytoplankton concentration at various concentrations of eggs and nauplii. The green line and data points show nauplii ingestion with egg concentration of 0 eggs/L. The black line and data points show nauplii ingestion with egg concentration of 50 eggs/L. The blue line and data points show nauplii ingestion with egg concentration of 1000 eggs/L. The red line and data points show nauplii ingestion with egg concentration of 3500 eggs/L.

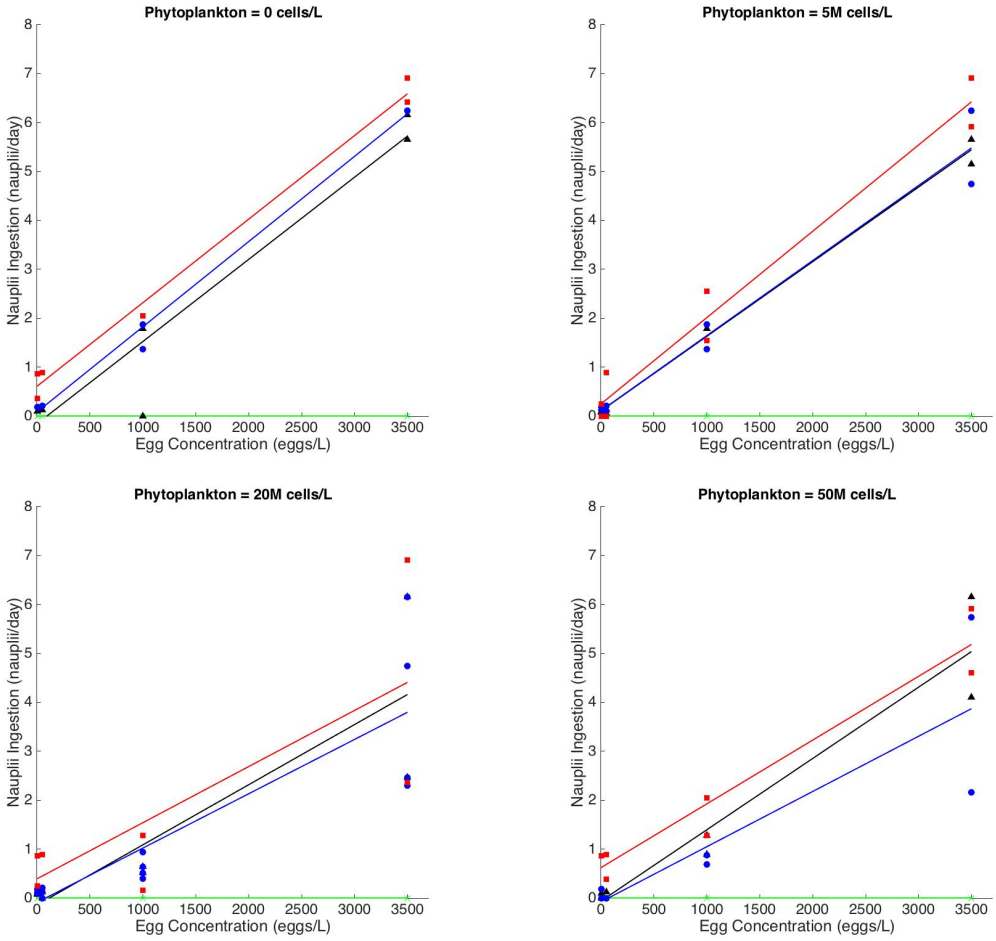


Figure A.6: Nauplii ingestion versus egg concentration at various concentrations of phytoplankton and nauplii. The green line and data points show nauplii ingestion with nauplii concentration of 0 nauplii/L. The black line and data points show nauplii ingestion with nauplii concentration of 15 nauplii/L. The blue line and data points show nauplii ingestion with nauplii concentration of 50 nauplii/L. The red line and data points show nauplii ingestion with nauplii concentration of 320 nauplii/L.

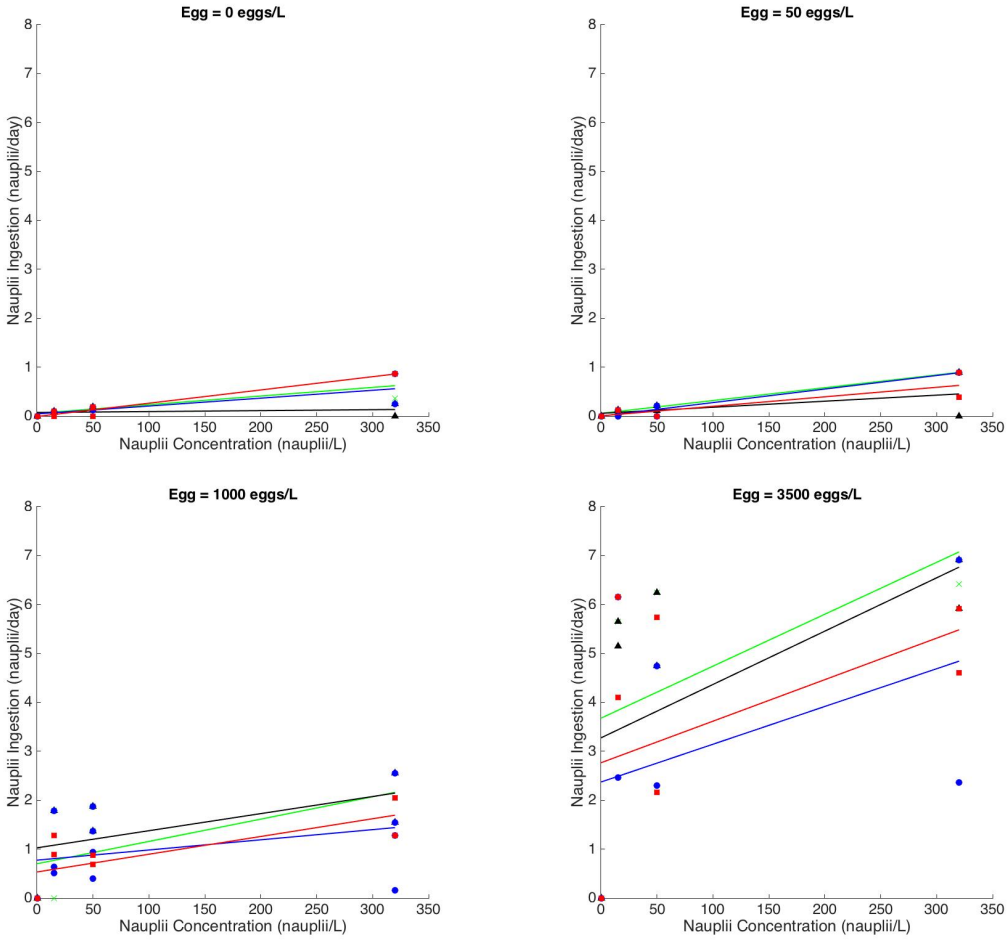


Figure A.7: Nauplii ingestion versus nauplii concentration at various concentrations of phytoplankton and eggs. The green line and data points show nauplii ingestion with phytoplankton concentration of 0 cells/L.The black line and data points show nauplii ingestion with phytoplankton concentration of 5×10^6 cells/L. The blue line and data points show nauplii ingestion with phytoplankton concentration of 2×10^7 cells/L. The red line and data points show nauplii ingestion with phytoplankton concentration of 5×10^7 cells/L.